REPORT DOCUMENTATION PAGE

Form Approved OMB Na. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response. Including the time for reviewing instructions, searching existing data sources,

tollection of information, including suggestions for re Davis Highway, Suite 1204, Arlington, VA 22202-4	ducing this burden to Washington He 302, and to the Office of Managem	edquarters Services, Directorate for It and and Budget, Paperwork Reduction	Vormation Operations and Reports, 1216 Jefferson Project (0704-0188), Washington, DC 20503.
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND	
	1994 - 1995	Final Report	_
4. TITLE AND SUBTITLE			5. FUNDING NUMBERS
Bioremediation of Oil S	pills by Whale M	icrobes	N00014-93-1-0823
Dr. A. Morrie Craig Daniel Wachenheim			
7. PERFORMING ORGANIZATION NAMES(8) Dr. A. Morrie Craig College of Veterinary Me Oregon State University 105 Magruder Hall Corvallis, OR 97331-480	edicine		8. PERFORMING ORGANIZATION REPORT NUMBER
8. SPONSORING / MONITORING AGENCY N. Office of Naval Research 800 North Quincy Street Arlington, VA 22217-566	1		10. SPONSORING / MONITORING AGENCY REPORT NUMBER
11. SUPPLEMENTARY NOTES			
a. DISTRIBUTION / AVAILABILITY STATEME	NT		12. DISTRIBUTION CODE
Distribution Unlimited			TIC QUALITY INSPECTED 2

13. ABSTRACT (Maximum 200 words)

Baleen whale forestomach anaerobic microbes were studied for their ability to detoxify recalcitrant environmental pollutants; these include components of crude oil and some chlorinated aromatic compounds which are constituents of oil spills not biodegraded by aerobic microbes. Bowhead whales have a forestomach similar to terrestrial ruminants in which are present large numbers of diverse bacteria and protozoa and which have been shown to degrade environmental contaminants. In this study, forestomach 'rumen' samples were collected on two occasions on the North Slope. When incubated with anthracene and naphthalene (PAH), these pollutants were degraded in the majority of sample sets. All simple aromatic hydrocarbons (benzine, toluene, xylenes, ethylbenzene) were degraded. PCBs were biodegraded by microbes from only two of the whales. Variable results were found with picric acid, trinitrotoluene (TNT), and atrazine, and were likely due to analytical difficulties. Anaerobic forestomach bacteria of whales, alone or in relationships with other microorganisms, represent a virtually untapped source of new degrading microorganism. Whale forestomach bacteria have adapted to an ecological niche where flow rates, mixing, and catabolism occur at rapid rates. These rates and the ability to metabolize complex molecules far exceed those of aerobic sediment and soil bacteria in biodegradation of environmental pollutants.

		15.	NUMBER OF PAGES	
Anaerobic microbes, bioremediation, detoxification,				
environmental pollutants, whales				
18. SECURITY CLASSIFICATION	19. SECURITY CLASSIFICATION	20.	LIMITATION OF ABSTRACT	
U U	OF ABSTRACT IT		IJŢ,	
	ants, whales	ants, whales 18. SECURITY CLASSIFICATION 19. SECURITY CLASSIFICATION	bioremediation, detoxification, ants, whales 18. SECURITY CLASSIFICATION 19. SECURITY CLASSIFICATION 20.	

Standard Form 298 (Ray, 2-89) Prescribed by ANISE Sad 229-18 298-102

FINAL REPORT

GRANT: # N00014-93-1-0823

PRINCIPAL INVESTIGATOR: A. Morrie Craig, Prof.

INSTITUTION: Oregon State University

Corvallis, OR 97331

GRANT TITLE: Bioremediation of Oil Spills by Whale Microbes

AWARD PERIOD: June 1994 - June 30, 1995

<u>OBJECTIVE</u>: To test whether anaerobic microbes found in whale forestomach fluid that can degrade recalcitrant compounds found in oil spills.

<u>APPROACH</u>: Bowhead whales, are baleen whales that consume roughly 2000 pounds of potentially pollutant laden krill with no toxic effects. It is proposed that whales have anaerobic microbes present in their forestomach similar to those found in terrestrial ruminants which have previously been shown to degrade environmental contaminants. We propose that the priority pollutants from the krill in the sea are detoxified by anaerobic microbes contained within the whale forestomach. Six forestomach fluid samples were collected on two occasions on the North Slope. Components of crude oil and some chlorinate aromatic compounds were incubated with this fluid to test their detoxifying effects.

ACCOMPLISHMENTS: Biodegradation of toxic compounds has been most intensively studied by using aerobic organisms. Much less is known about the degrading potential of anaerobic organisms. The forestomach is a specialized organ present in many terrestrial animals (cattle, sheep, goats, and llamas) in addition to baleen whales. The forestomach contains a huge diversity of bacteria, many of which are capable alone or in relationships with other microorganisms to degrade a wide range of naturally occurring compounds. Recent evidence suggests that these same bacteria can degrade many synthetic compounds that are known to be recalcitrant under typical conditions. Because forestomach bacteria have adapted to an ecological niche where flow rates, mixing, and catabolism occur at rapid rates, these organisms have a potential to biodegrade environmental pollutants at faster rates than sediment and soil bacteria. Anaerobic forestomach bacteria represent a virtually untapped source of degrading potential.

Findings from this study show that whale forestomach anaerobic microbes are able to detoxify recalcitrant environmental pollutants, in particular, components of crude oil and some chlorinated aromatic compounds. Anthracene and naphthalene (PAH) incubated with the forestomach fluid were found to be degraded. All simple aromatic hydrocarbons (benzine, toluene, xylenes, and ethylbenzene) were also found to be degraded by the whale microbes. PCBs were degraded by only two of the whale samples. Results on picric acid, trinitrotoluene (TNT), and atrazine gave mixed results from the different whales, but this is most likely due to analytical difficulties. Cholestan, the hopane fraction from bunker fuel, was analyzed by EPA at Gulf Breeze.

<u>CONCLUSIONS</u>: Anthracene, naphthalene, PCBs, aromatic compounds, and, potentially, hopanes appear to be degraded by anaerobic microbes from the forestomach of baleen whales. Therefore, anaerobic microbes show promise for cleaning up oil spills.

<u>SIGNIFICANCE</u>: Our studies provide further tools which can be employed to detoxify and prevent environmental contamination. These microbes are capable of being grown in large vats. The anaerobes can then be encapsulated for protection against oxygen. These microbes could ultimately be engineered into controlled-release capsules that could be distributed over oil spills to eliminate their environmental contamination.

PATENT INFORMATION: None.

AWARD INFORMATION: None.

PUBLICATIONS AND ABSTRACTS:

- 1. Leedle JAZ, Lotrario JB, Hovermale JT, Craig, AM. Forestomach anaerobic microflora of the bowhead whale (*Balaena mysticetus*). <u>95th General Meeting, American Society of Microbiologists</u>, Washington, DC, May 1995. Abstract and poster.
- 2. Hovermale JT, Lotarario JB, Leedle JAZ, Blythe LL, Craig, AM. A unique source of anaerobic microorganisms with biodegradation potential. <u>95th General Meeting, American Society of Microbiologists</u>, Washington, DC, May 1995. Abstract and poster.

(Following grant period)

- 1. Hovermale JT, Lotrario JB, Leedle JAZ, Blythe LL, Craig AM. A unique source of anaerobic microorganisms that degrade crude oil. <u>Gordon Conference</u>, New Hampton, New Hampshire, July 1995. Poster.
- 2. Craig AM. Whale microbes that degrade oil spill components. <u>Proceedings, International Congress of Clinical Chemistry</u>, London, England, July 1996. Abstract.